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ORIGINAL ARTICLE

## Environment-specific shell shape variation in the boring mytilid *Leiosolenus patagonicus*

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### ABSTRACT

Environmental conditions induce phenotypic responses (behavioural, morphological and physiological) in many marine species. The boring mytilid *Leiosolenus patagonicus* inhabits different types of substrata, such as sandstone intertidal and hard subtidal substrata (here called 'lifeless-substratum') and shells of bivalve species (here called 'live-substratum'), where they are exposed to different restrictions in their growth. We used geometric morphometric methods to compare the contour shell shapes from each type of substratum (live and lifeless) since we expected the body shape to differ between individuals from these different substrata. The results showed that the shell shape depends on the type of substratum where the larvae recruit. The mean shell shapes of individuals from the live-substratum are more slender than those of the individuals growing inside the lifeless-substratum. Individuals from live-substratum can adapt their phenotype depending on the oyster's anti-parasitism responses, while in lifeless-substratum they are able to build their own refuges.

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### Introduction

A variety of marine boring bivalves dwell in holes that they make in hard substrata such as sedimentary rock, wood, coral and other mollusc shells (Ruppert & Barnes 1994). In adopting this lifestyle, these boring bivalves erode the hard substratum in order to be protected from wave and current action or from predatory pressures (Gosling 2008). Physical conditions (e.g. substratum type, salinity, temperature) as well as ecological traits (e.g. competitive interactions, predation pressure, feeding behaviour) may differ among different environments (Futuyama 2005). Because of this environmental heterogeneity, notable phenotypic variations may exist among individuals with the same genotype from different sites (Miner et al. 2005; Sánchez et al. 2011). Therefore, organisms that recruit in different environmental conditions may exhibit behavioural, physiological and morphological differences (Pigliucci 1996; Hollander et al. 2006).

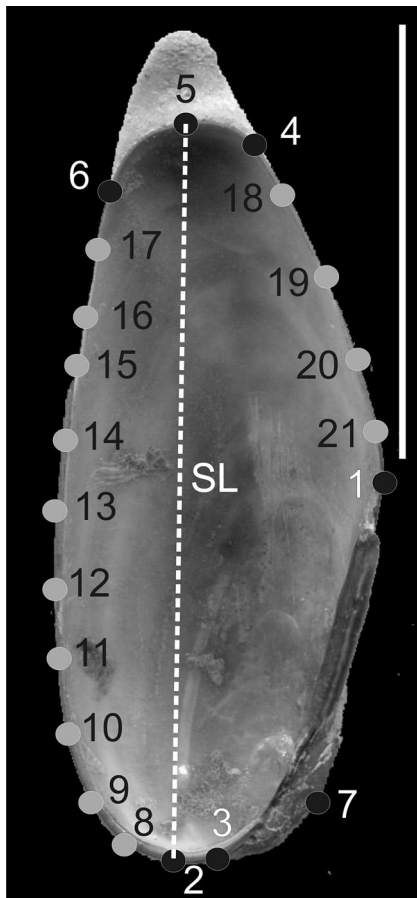
The boring mytilid *Leiosolenus patagonicus* (d'Orbigny, 1842) inhabits intertidal and shallow waters in the South-western Atlantic from Santa Catarina state (Brazil, 28°S) to Tierra del Fuego (Argentina, 55°S) (Pastorino 1995; Rios 2009; Rosenberg 2009). Because it actively bores the substratum where it lives, it is

classed as a euendolith (Golubic et al. 1981). On Patagonian coasts, *L. patagonicus* bores flask-shaped burrows into rocky intertidal and subtidal sediments, as well as the shells of several bivalve species, being considered the most harmful borer organism on native puelche oyster, *Ostrea puelchana* d'Orbigny, 1842 (Diez et al. 2014). These authors have reported a decrease in the condition index of the oyster due to the boring activity of *L. patagonicus*. Because of environmental differences among these substrata, we expect the body shape to differ among individuals from each type of substratum because of habitat-specific adaptation. Specifically, we expect that there are differences between shell shapes from the different types of substrata and between intertidal and subtidal habitats. Particularly, we predict that the specimens inhabiting the shell of the bivalves have a smaller (Bagur et al. 2013) and more slender shell shape compared with that of individuals from lifeless-substrata.

### Materials and methods

A total of 248 individuals were collected from two types of substrata, defined as live-substratum (bivalve shells) and lifeless-substratum (sandstone and hard substrata)

from intertidal and subtidal areas of the Fracasso Beach (42°25'S, 64°07'W – San José Gulf, Argentina). The distance between sample sites was not more than 4 km. Shell length (SL) was measured as the straight line between landmarks 2 and 5 (Figure 1). Fifty-one individuals were collected manually from sandstone substrata of the intertidal (SL: 10.32–39.93 mm); the rest of the samples were collected from a 15 m deep area by scuba diving: 77 from lithified siltstones with carbonatic content, Puerto Madryn Formation (late Miocene, about 10 Ma), henceforth: hard substratum (SL: 10.11–38.73 mm), 71 from oyster shells (*Ostrea puelchana*, SL: 4.98–19.74 mm) and 49 from the shells of jingle oysters (*Pododesmus rudis* (Broderip, 1834), also called “false oyster” (FO), SL: 4.28–18.30 mm).



**Figure 1.** Diagram showing the position of the seven landmarks (black dots) and 14 semi-landmarks (grey dots) used to define the shell shape of *Leiosolenus patagonicus*. These landmarks are: (1) ligament, (2) maximum posterior curvature, (3) limit with the umbo and start of posterior curvature, (4) dorsal limit with anterior curvature, (5) internal anterior end (6) ventral limit with anterior curvature, (7) dorsal margin, (8–17) semi-landmarks along the ventral outline between landmarks 2 and 6, (18–21) semi-landmarks along the anterior-dorsal outline between landmarks 4 and 1. SL: Shell length (dotted line). Scale bar = 1 cm.

The shells were dissected, carefully cleaned and when dry, they were numbered and scanned with the inner surface parallel to the plane of scanning using an Epson Perfection v350 scanner with a resolution of 600 dpi. Analyses of the shell shape were performed using geometric morphometric (GM) techniques of landmarks and semi-landmarks. We used GM instead of classic morphometrics because it presents some advantages, such as the fact that the size and shape can be analysed separately and the results of multivariate analysis can be visualized in a graphic way (observing both the magnitude as well as the direction of change) since the implicit nature of geometric shape information is not lost during the analysis (Adams et al. 2004). The shape of the individuals was captured by using the Cartesian coordinates of a two-dimensional configuration of anatomical landmarks and semi-landmarks (Figure 1). The semi-landmarks were aligned using TPSrelw software (Rohlf 2003b) to calculate the algorithm that extends the thin plate splines and warp analysis to the sliding semi-landmark points proposed by Bookstein (1996). In this method, the semi-landmark points are slid along the outline curve in order to minimize the bending energy of the configurations (Gunz et al. 2005; Klingenberg 2008). All specimens were digitized by one observer (AF) using TPSdig2 software (Rohlf 2003a). The landmark configuration was superimposed by generalized Procrustes analysis (Rohlf & Slice 1990; Slice et al. 1996). This procedure translates and rotates the landmark configurations to a common origin and scales them to unit centroid size.

One-way ANOVA ( $\alpha = 0.05$ ) in the software program INFOSTAT (Di Rienzo et al. 2012) was used to detect the presence of statistically different groups in the shell size. Post hoc Tukey ( $\alpha = 0.05$ ) tests were used when ANOVA found significant differences between substrata.

The shape/size variation, called allometry, refers to a change in shape associated with size differences. To assess and control putative allometric effects, we computed pooled within-substrata multivariate regression of shape (Procrustes coordinates used as dependent variables) on size (centroid size used as independent variable) (Bookstein 1991; Monteiro 1999; Klingenberg 2011). The centroid size was used as a proxy for shell size and calculated for each specimen as the square root of the sum of the squared deviations of landmarks from the centroid (Bookstein 1991; Zelditch et al. 2004). To evaluate the independence between the shape and size variables, we ran a permutation test with 1000 rounds (Good 2000). To study the magnitude and direction of shell shape variation, a principal component

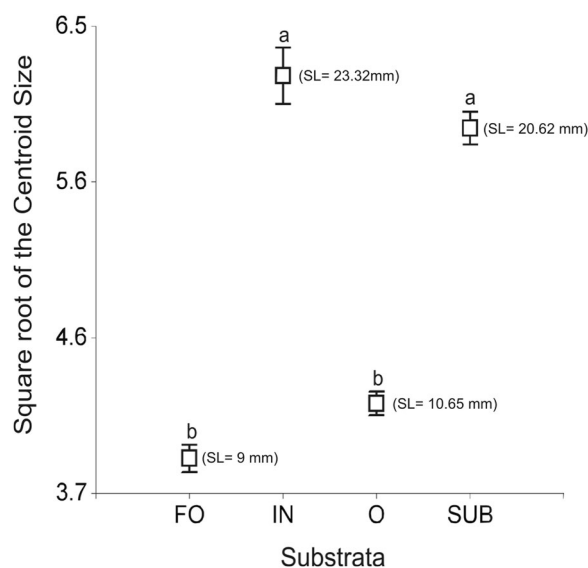
analysis (PCA) of the variance-covariance matrix (Zelditch et al. 2004) was done. The maximum differences in shell shape separating substrata were assessed by a Canonical Variate Analysis (CVA). Furthermore, to assess the Mahalanobis distances (shape distances) and identify different groups, we used the Multivariate Di Rienzo, Guzman, Casanoves (MDGC) method, an extension of the multivariate case of a multiple comparison method based on cluster analysis generated using an unweighted pair-group method with arithmetic mean (UPGMA; Valdano & Di Rienzo, 2007).

## Results

Size of the individuals (square root of the centroid size) differed significantly between lifeless and live substrata ( $F_{3,244} = 110.71$ ,  $P < 0.0001$ ; Figure 2).

Growth pooled within-substrata of the shell of *L. patagonicus* was allometric. The multivariate regression of shape on centroid size was significant (permutation test with 1000 random permutations,  $P < 0.0001$ ), and accounted for 5.2% of the total amount of shape variation. The main shell form variation was related to the size and shape of the umbo and antero-dorsal development (Figure 3).

After this test, we focused on shell shape variations; thus, for subsequent analyses we used the regression



**Figure 2.** Square root of the centroid size (mean  $\pm$  SE) as an estimator of shell size of *Leiosolenus patagonicus* from different substrata. Letters above the bars represent statistical significance of pairwise comparisons (different letters show significant differences at  $P < 0.05$ ). Code for groups: SUB, subtidal (hard substratum); IN, intertidal (sandstone substratum); O, *Ostrea puelchana* and FO, *Pododesmus rudis* (subtidal live-substratum). Mean of shell length (SL) for each group is shown in parentheses.

residual as a new size-unrelated shell shape variable. The pattern of the shell shape variation is summarized in Figure 4 with scatterplots for the first 2 PCs of shape. The specimens from the four groups were mostly overlapped. The geometric interpretation of the positive extremes of PC1 was associated with the development, in the positive values, of the anterior and posterior edge of the shell showing a slender shape (with a narrow dorsal and ventral edge). The second PC axis was related to the development of the anterior part of shell shape (with a shorter posterior part than the anterior one) and elongation of the posterior edge (Figure 4).

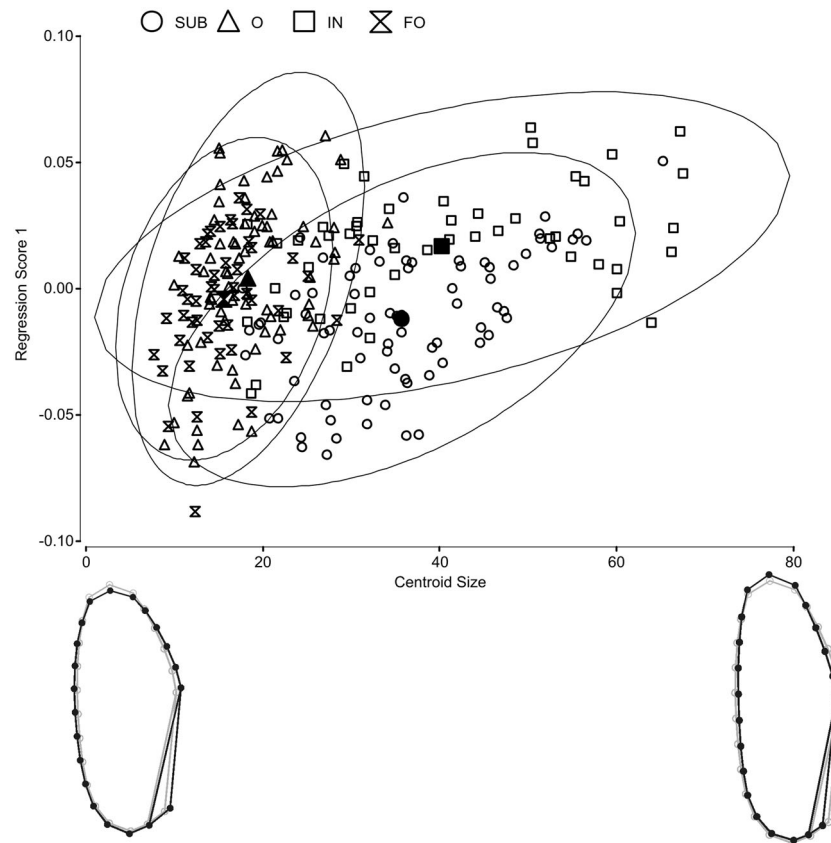
The shell shape variations among the groups sampled were successfully discriminated using CVA of the residuals from the regression of shape on centroid size. The first two canonical axes (CV) explained 80% of the total variance. The greatest difference in CV1 was between live- and lifeless-substrata, being associated with a more slender and elongated shell shape in the latter. The CV2 was mostly associated with the narrowing and enlarging anterior part of the shell (Figure 5a), and this variation was observed between intertidal vs subtidal substrata. Comparison of mean shape among groups indicates there are significant differences in shell shape among them.

The dendrogram calculated on the Mahalanobis distance matrix showed that, even though each group had a shell shape with statistically significant differences ( $P < 0.05$ ), the type of substratum (live and lifeless) was the main grouping factor (Figure 5b).

## Discussion

As expected, the results obtained in the present study showed significant differences in the shell size and shape of the boring mytilid *Leiosolenus patagonicus* inhabiting different types of substrata: live and lifeless. Bagur et al. (2013) showed that different types of substrata generate different restrictions on the growth of individuals of this species. In the present study, the bivalve shells could be the main environmental constraints to the shell form. The detection of allometry, the relation between size and shape, indicates that the shape variations were dependent on the size variation. The main shell form variation related to smaller individuals (from live-substratum) was associated with size and shape of umbo and antero-dorsal development edge. For a real shape study, we controlled the allometric effect by using the regression residual as a new size-unrelated shell shape variable.

Organisms frequently develop specialized phenotypes adapted to local environmental conditions



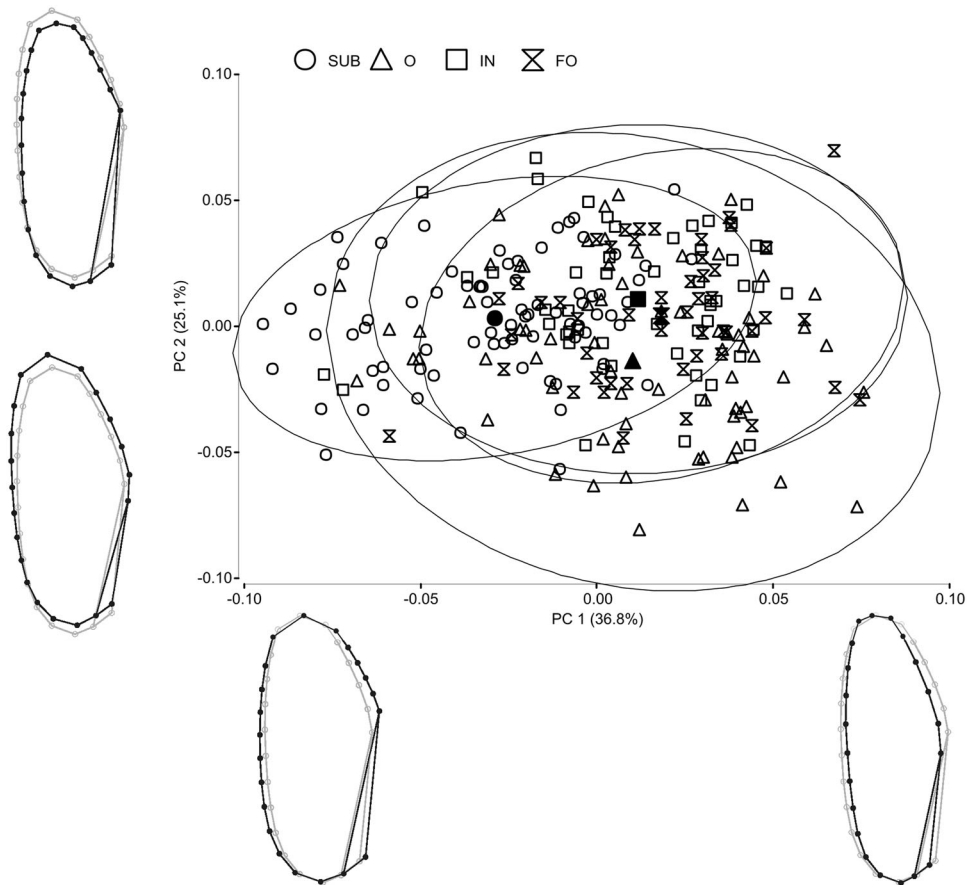
**Figure 3.** Pooled within-substrata regression of shape onto size: shape scores as a function of centroid size illustrating allometric growth of the different *Leiosolenus patagonicus* growing in different substrata. Shapes at the opposite extremes of the range of allometric variation are shown by using vector diagrams from the black dot diagram (mean shape), indicating the predicted landmark shift corresponding to an increase of centroid size by a 30 scale factor. See Figure 2 for reference labels for the groups.

(Postma & Van Noordwijk 2005). The use of geometric morphometric analysis allowed us to characterize the shell shape variations of substrata types and to determine the relationships among the shell shapes of groups of *L. patagonicus*. The major difference between *L. patagonicus* individuals from the two types of substrata (live and lifeless) was that the more slender and elongated shell shape and smaller size were recorded in samples from live-substratum. Diez et al. (2014) have reported that the presence of the boring mytilid *L. patagonicus* inhabiting *Ostrea puelchana* shells caused a decrease in the condition index of the oyster, probably due to the metabolic energy costs to the host of the production of extra-organic matter (conchiolin) to seal off the holes. In this type of habitat, the growth of *L. patagonicus* could be constricted by the defence mechanism of the host bivalve, causing a physical compression.

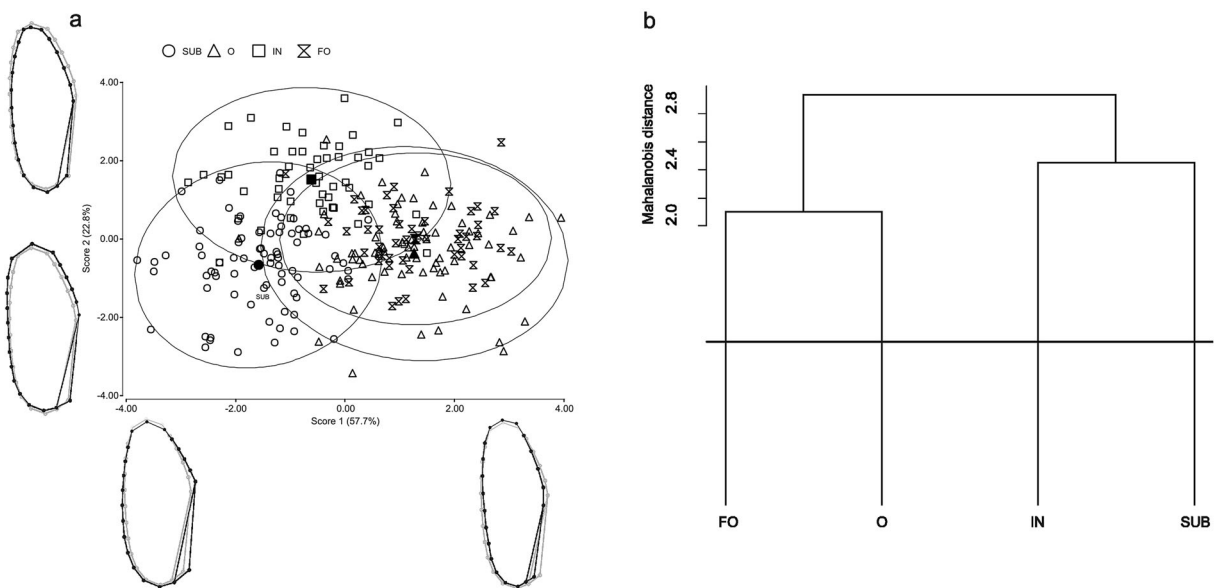
The other important shell shape variation was associated with the physical conditions from intertidal vs subtidal habitats. Patagonian intertidal rocky shore communities are exposed to unusually harsh physical conditions, and consequently they are strongly

structured by physical stress (Bertness et al. 2006). In contrast, subtidal habitats are more homogeneous and stable, as they lack the direct action of wave splash and show less temperature variation compared with intertidal shores. Shells from intertidal substrata had a narrower and longer shape in the anterior part of the shell than those from subtidal substratum. This particular shell shape could be related to the temperature variations, desiccation risk and wave exposure, mainly registered in this kind of environment.

In summary, we point out that the variance of shell shape of *L. patagonicus* individuals is due to the bivalve host restriction, since an *L. patagonicus* that settles on live-substratum has to adapt its phenotype depending on the bivalve responses, while in lifeless-substratum it is able to build its own refuge. However, the body shape is a complex trait generally influenced by multiple environmental variables in addition to other evolutionary factors (Langerhans et al. 2007). In our study, the results support the hypothesis that the principal constraint is the type of substratum where larvae settle, and the secondary effect on shell shape is due to the influence of physical factors in both intertidal



**Figure 4.** Plot of the two principal components (PCs) for different substrata groups based on Procrustes distances. The figures represent the displacement vectors from the overall mean shape (grey dot) to the positive and negative extreme shape (vector) for each PC. Shape changes have a scale factor of 0.1. Percentages of explained variance for each axis are in parentheses. See Figure 2 for reference labels for the groups.



**Figure 5.** (a) Analysis of the maximum shell shape variation along the first two canonical axes and transformation grid diagrams show shape changes from mean shape (grey dot) to the positive and negative extreme (black dot, scale factor of 10) in both axes. The largest symbols indicate the average for each group. (b) UPGMA dendrogram showing the relationships among shell shapes from different substrata. The cut-off criterion ( $P = 0.05$ ) obtained with the MDGC test is indicated with a horizontal line. See Figure 2 for reference labels for the groups.

and subtidal habitats. Therefore, the different morphotypes of *L. patagonicus* might be distinguished according to the type of substratum and habitat where it settles, showing a high degree of intra-specific variability.

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## Disclosure statement

No potential conflict of interest was reported by the authors.

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